Treatment of atrial fibrillation with doxapram: TASK-1 potassium channel inhibition as a novel pharmacological strategy

Felix Wiedmann¹,²,³, Christoph Beyersdorf¹,³, Xiao-Bo Zhou²,⁴, Manuel Kraft¹,²,³, Amelie Paasche¹,³, Natasa Jávorszky¹,³, Susanne Rinné⁵, Henry Sutanto⁶, Antonius Büscher¹,²,³, Kathrin I. Foerster⁷, Antje Blank⁷, Ibrahim El-Battrawy²,⁴, Xin Li⁴, Siegfried Lang²,⁴, Ursula Tochtermann⁸, Jamila Kremer⁸, Rawa Arif⁸, Matthias Karck⁸, Niels Decher⁵, Gunther van Loon⁹, Ibrahim Akin²,⁴, Martin Borggrefe²,⁴, Stefan Kallenberger¹⁰, Jordi Heijman⁶, Walter E. Haefeli⁷, Hugo A. Katus¹,²,³, Constanze Schmidt¹,²,³*,

Affiliations:
¹Department of Cardiology, University of Heidelberg, Heidelberg, Germany; ²DZHK (German Center for Cardiovascular Research), partner site Heidelberg / Mannheim, University of Heidelberg, Heidelberg, Germany; ³HCR, Heidelberg Center for Heart Rhythm Disorders, University of Heidelberg, Heidelberg, Germany; ⁴First Department of Medicine, University Medical Center Mannheim, Mannheim, Germany; ⁵Institute for Physiology and Pathophysiology, Vegetative Physiology and Marburg Center for Mind, Brain and Behavior MCMBB, University of Marburg, Marburg, Germany; ⁶Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, Maastricht, The Netherlands; ⁷Department of Clinical Pharmacology and Pharmacoepidemiology, University Hospital Heidelberg, Heidelberg, Germany; ⁸Department of Cardiac Surgery, University Hospital Heidelberg, Heidelberg, Germany; ⁹Department of Large Animal Internal Medicine, Equine Cardioteam, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium; ¹⁰Digital Health Center, Berlin Institute of Health (BIH) and Charité, Berlin, Germany and Health Data Science Unit, University Hospital Heidelberg, Heidelberg, Germany.

Short Title: Treatment of AF with the TASK-1 blocker doxapram

*Corresponding author:
Prof. Dr. med. Constanze Schmidt, FEHRA, FESC
Department of Cardiology
Medical University Hospital Heidelberg
Im Neuenheimer Feld 410
D-69120 Heidelberg, Germany
Tel.: ++49 6221 568187
Fax: ++49 6221 565724
E-Mail: Constanze.Schmidt@med.uni-heidelberg.de

Total word count of the manuscript: 8,936

Manuscript category: Original research, translational cardiac electrophysiology

Subject codes: Arrhythmias; Atrial Fibrillation; Electrophysiology; Ion Channels/Membrane Transport; Translational Studies.
1. Abstract

**Aims:** TASK-1 ($K_{\text{2P}}3.1$) two-pore domain potassium channels are atrial-specific and significantly upregulated in atrial fibrillation (AF) patients, contributing to AF-related electrical remodelling. Inhibition of TASK-1 in cardiomyocytes of AF patients was shown to counteract AF-related action potential duration shortening. Doxapram was identified as a potent inhibitor of the TASK-1 channel. In the present study, we investigated the antiarrhythmic efficacy of doxapram in a porcine model of AF.

**Methods and Results:** Doxapram successfully cardioverted pigs with artificially induced episodes of AF. We established a porcine model of persistent AF in domestic pigs via intermittent atrial burst stimulation using implanted pacemakers. All pigs underwent catheter-based electrophysiological investigations prior to and after 14 d of doxapram treatment. Pigs in the treatment group received intravenous administration of doxapram once per day. In doxapram-treated AF pigs, the AF burden was significantly reduced. After 14 d of treatment with doxapram, TASK-1 currents were still similar to values of sinus rhythm animals. Doxapram significantly suppressed AF episodes and normalized cellular electrophysiology by inhibition of the TASK-1 channel. Patch-clamp experiments on human atrial cardiomyocytes, isolated from patients with and without AF could reproduce the TASK-1 inhibitory effect of doxapram.

**Conclusions:** Repurposing doxapram might yield a promising new antiarrhythmic drug to treat AF in patients.
2. Translational perspective

Pharmacological suppression of atrial TASK-1 potassium currents prolongs atrial refractoriness with no effects on ventricular repolarization, resulting in atrial-specific class III antiarrhythmic effects. In our preclinical pilot study the respiratory stimulant doxapram was successfully administered for cardioversion of acute AF as well as rhythm control of persistent AF in a clinically relevant porcine animal model.

3. Keywords

Antiarrhythmic pharmacotherapy; atrial fibrillation; doxapram; rhythm control; TASK-1.

4. Non-standard Abbreviations and Acronyms

AERP, atrial effective refractory period; AF, atrial fibrillation; AP, action potential; APD, action potential duration; AV node, Atrioventricular node; AVRP, atrioventricular node refractory period; BCL, basic cycle length; cAF, chronic atrial fibrillation; CI, confidence interval; cSNRT, corrected sinus node recovery time; ECG, electrocardiogram; EP, electrophysiology; i.v., intravenous; K$_{2p}$, two-pore-domain potassium; SACT, sinoatrial conduction time; SNRT, sinus node recovery time; SR, sinus rhythm; TASK, TWIK-related acid-sensitive K$^+$ channel; TWIK, tandem of P domains in a weak inward rectifying K$^+$ channel; VERP, ventricular effective refractory period.
5. Introduction

Atrial fibrillation (AF) is by far the most common sustained cardiac arrhythmia\(^1\). Safe and effective pharmacological treatment of AF, however, still remains an unmet medical need. In the western world, approximately three percent of the population suffer from AF\(^2\). Its prevalence and incidence increase with age. Consecutively, it can be foreseen that the number of patients suffering from AF will increase in our aging population\(^3\). The effectiveness of current pharmacological and interventional strategies against AF is still suboptimal resulting in an urgent need for novel therapeutic approaches\(^4\).

The TASK-1 (TWIK [tandem of P domains in a weak inward rectifying K\(^+\) channel] - related acid-sensitive K\(^+\) channel; K\(_{2P}\)3.1) potassium channel is a member of the two-pore-domain potassium (K\(_{2P}\)-) channel family\(^5, 6\). In the human heart, TASK-1 (hK\(_{2P}\)3.1) is specifically expressed in the atria. Recently, it was described that TASK-1 expression is upregulated in patients suffering from AF, contributing to the pathological shortening of the atrial action potential duration (APD)\(^7, 8\) together with calcium handling abnormalities, increased repolarizing potassium currents, like \(I_{K1}\) as well as agonist-independent ‘constitutive’ \(I_{K,ACH}\), and alterations in cell-to-cell electrical coupling\(^9-13\).

Therefore, TASK-1 channels represent a potential novel target for pharmacological rhythm control by counteracting AF-induced APD shortening. The TASK-1 current can be inhibited by experimental ion channel inhibitors as well as clinically used antiarrhythmic drugs\(^14-17\). For example, pharmacological inhibition of TASK-1 using the experimental inhibitor A293 was recently shown to exhibit atrial specific antiarrhythmic properties in our porcine large animal model\(^18, 19\).

The pyrrolidinone derivative doxapram, clinically employed as a respiratory stimulant for over half a century, was recently identified as a potent inhibitor of TASK-1 channels\(^20-23\). Doxapram acts on both brainstem respiratory centers and peripheral carotid chemoreceptors, where
TASK-1 and TASK-3 channels are abundantly expressed\textsuperscript{24}. Carotid body type 1 cells are regulated via oxygen-sensitive $K^+$ currents which are most likely carried by TASK channels and doxapram probably acts by inhibiting these channels\textsuperscript{22, 24}. IC$_{50}$ values of doxapram on TASK-1 are well within its therapeutic range, and thus this FDA- and EMA-approved drug might bear the potential to be used for rhythm control in AF patients.

Our preclinical trial aimed to assess the antiarrhythmic potential of pharmacological TASK-1 channel inhibition by doxapram using clinically relevant large animal models of acute episodes of paroxysmal AF and persistent AF. Here, the respiratory stimulant doxapram was successfully applied for cardioversion of paroxysmal AF episodes as well as rhythm control of persistent AF. Additionally, patch-clamp experiments on isolated human atrial cardiomyocytes from patients with AF could confirm an efficient antiarrhythmic effect of doxapram. Finally, the antiarrhythmic potential of TASK-1 inhibition by doxapram could be reproduced by multiscale \textit{in silico} modelling of human atrial electrophysiology. Altogether, these studies provide a clear rationale for the DOCTOS trial (doxapram conversion to sinus rhythm; EudraCT No: 2018-002979-17) which investigates whether doxapram can cardiovert AF in patients.
6. Methods

For a detailed description of the employed methodology please refer to the online supplement.

6.1 Porcine AF model

Induction of persistent AF in pigs was carried out by atrial burst pacing via an implanted cardiac pacemaker (St. Jude Medical, St. Paul, MN, USA). To prevent tachycardia-induced heart failure, atrioventricular (AV) node ablation was performed under fluoroscopic guidance. Acute AF was induced via right-atrial burst stimulation (400–1,200 min\(^{-1}\)) during electrophysiological studies. Animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health (NIH publication No. 86-23, revised 1985), with EU Directive 2010/63/EU, and with the current version of the German Law on the Protection of Animals.

6.2 Patients

The study protocol involving human tissue samples was approved by the ethics committees of the Medical Faculty of Heidelberg University (Germany; S-017/2013). Written informed consent was obtained from all patients and the study was conducted in accordance with the Declaration of Helsinki. A total of 12 patients with sinus rhythm (SR) or chronic AF (cAF) undergoing open heart surgery for coronary artery bypass grafting, heart valve repair or valve replacement were included in the study.

6.3 Cellular electrophysiology

Human and porcine atrial myocytes were isolated freshly as described in the online supplement. Electrophysiological recordings were carried out at room temperature (22–23°C) using the whole-cell patch-clamp configuration.

6.4 Molecular biology and Xenopus oocyte electrophysiology
Copy RNA was synthesized, using the mMESSAGE mMACHINE T7 Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA) and injected into stages V and VI defolliculated *Xenopus laevis* oocytes as described earlier\textsuperscript{7}. 

7. Results

7.1 Doxapram inhibits human and porcine TASK-1 channels

Major anatomical and physiological analogies to humans constitute specific advantages of using pigs as a large animal model in cardiovascular research. Human (h) and porcine (p) TASK-1 channels display a large degree of homology, resulting in comparable functional and regulatory properties\textsuperscript{18,25}. \textit{In silico} docking simulations of doxapram into the intracellular pore of an open-state pTASK-1 homology model based on the recently revealed crystal structure of hTASK-1 (PDB ID: 6RV2)\textsuperscript{26} predicted that its binding site is located in close proximity to the pore-lining amino acids T93, L122, T199, and L239 (Fig. 1 A to C). It was experimentally shown that these amino acid residues contribute to the drug binding site of the high affinity TASK channel blocker A293\textsuperscript{27} and likewise mediate doxapram binding\textsuperscript{21,23}. A comparison of the structure and predicted drug binding site within the TASK-1 inner channel pore is presented in Online Figure I.

Following heterologous expression in \textit{Xenopus oocytes}, human and porcine TASK-1 channel subunits give rise to comparable macroscopic currents (Fig. 1 D and G). Outward potassium currents were measured using the two electrode voltage-clamp technique \textit{24 h} to \textit{72 h} after injection of copy RNA encoding either human or porcine TASK-1 orthologs. Currents were elicited by application of depolarizing test pulses (500 ms), applied in 20-mV increments from a holding potential of -80 mV to +60 mV (0.2 Hz, see Fig. 1 D and G). Current amplitudes were measured at the end of the +20 mV pulse. After a control period with no significant amplitude changes (10 min), administration of doxapram (10 µmol/l, 30 min) resulted in rapid decline of outward potassium currents (human: 71.8 ± 3.6 %, $n = 7$, $P = 0.0002$; porcine: 73.4 ± 8.0 %, $n = 4$, $P = 0.003$; Fig. 1 D to I). Doxapram blockade did not affect the current voltage relationship of human or porcine TASK-1 orthologs. Inhibition of TASK-1 currents by doxapram was concentration-dependent (Fig. 1 F and I). Half-maximal
concentrations (IC$_{50}$) of human and porcine TASK-1 by doxapram were 0.88 μmol/l (1σ-confidence interval [CI], [0.46 μmol/l, 1.07 μmol/l]) and 0.93 μmol/Ll (1σ-CI, [0.43 μmol/l, 1.31 μmol/l]) ($n = 3–24$ cells per concentration step).

Among human atrial potassium channels, doxapram (5 μmol/l) displayed specific TASK channel inhibition without significantly affecting heterologously expressed TASK-4, TREK-2, Kir2.1, Kir3.1/Kir3.4, Kv1.4, Kv1.5, Kv2.1, Kv4.3, MaxiK, Nav1.5 or Cav1.2 channels (Fig. 1 J and K). TASK-3 channels were blocked by doxapram with similar efficiency as TASK-1. However, our former studies pointed towards negligible cardiac expression levels of TASK-3. Application of 100 μmol/l doxapram was associated with mild to moderate off target effects on Kv1.4, Kv2.1 and hERG currents (Online Figure II).

7.2 In vivo antiarrhythmic properties of doxapram in a porcine large animal model

Upon acute intravenous (i.v.) administration of doxapram (2 mg/kg body weight) in healthy control pigs ($n = 3$ German landrace pigs of both sexes), a rapid peak and decline of doxapram plasma levels could be observed (Fig. 2 A). Surface electrocardiograms (ECGs), recorded 5 min after i.v. administration of doxapram (2 mg/kg body weight), did not show relevant alterations in PQ, QRS, QT or QTc intervals compared to baseline conditions ($n = 7$ independent experiments performed on $N = 4$ individual pigs; Fig. 2 B). A trend towards increased heart rates was observed after application of doxapram (1 mg/kg body weight: 15.9 % increase; 2 mg/kg body weight 20.2 % increase; Fig. 2 C). Invasive blood pressure recordings performed in anesthetized pigs revealed a slight trend towards increased systolic, diastolic, and mean blood pressure levels under doxapram treatment that did not reach statistical significance ($n / N = 7 / 4$; Fig. 2 D). Atrial effective refractory periods (AERPs) measured at different basic cycle lengths (BCL), were significantly prolonged upon TASK-1 inhibition by doxapram consistent with class-III antiarrhythmic effects (Fig. 2 E). The AERP measured at a BCL of 500 ms ($\text{AERP}_{500}$) increased from $216.4 \pm 24.4$ ms under control
conditions to $251.4 \pm 22.1$ ms after administration of 1 mg/kg body weight doxapram, and
further increased to $254.7 \pm 19.7$ ms after 2 mg/kg body weight doxapram ($P = 0.0011$ and
$P = 0.0036$; $n/N = 6/4$; Fig. 2 E). The $\text{AERP}_{400}$ was prolonged from $212.1 \pm 19.8$ ms to
$232.9 \pm 18.4$ ms after i.v. injection of 1 mg/kg body weight doxapram, and to $237.9 \pm 20.9$ ms
after i.v. injection of 2 mg/kg body weight doxapram ($P = 0.029$ and $P = 0.00026$; $n/N = 6/4$;
Fig. 2 E); the $\text{AERP}_{300}$ was prolonged from $170.0 \pm 15.7$ ms to $205.0 \pm 13.5$ ms at 1 mg/kg
body weight doxapram, and to $212.5 \pm 23.5$ ms at 2 mg/kg body weight doxapram ($P = 0.0094$
and $P = 0.046$, respectively; $n/N = 6/4$; Fig. 2 E).

7.3 Pharmacological cardioversion of acute paroxysmal AF episodes by i.v. doxapram

treatment

Subsequently, the antiarrhythmic potential of doxapram-induced prolongation of AERP was
studied in a porcine model of acute episodes of paroxysmal AF. AF episodes were artificially
induced by right-atrial burst pacing (10 V, 150 ms to 50 ms cycle length, 2 to 8 s duration)
during electrophysiological studies. Following a control period of 5 min to confirm stability of
the induced AF episode, pigs were treated with different concentrations of doxapram (Fig. 2 F).
Whereas doxapram doses of 1 mg/kg body weight (empty circles) did not result in
cardiowersion of AF during an observation period of 10 min, strikingly, doxapram doses
$\geq 1.5$ mg/kg body weight (blue dots) resulted in a 100 % cardioversion rate. After i.v.
administration of doxapram, the time to cardioversion was monitored in surface and
intracardiac ECGs. The average time to cardioversion was $2.5 \pm 0.6$ min after i.v.
administration of doxapram ($n = 17$; Fig. 2 G). On average, doses of 1.85 mg/kg body weight
were sufficient for cardioversion ($n = 17$; Fig. 2 H).


7.4 Effects of continuous doxapram treatment in a porcine model of persistent AF

To study the efficacy of pharmacological TASK-1 inhibition by doxapram in rhythm control, doxapram was administered daily in a porcine model of persistent AF. In all animals, echocardiography and invasive electrophysiological studies were conducted (Online Figure III), a dual chamber pacemaker was implanted, and an AV node ablation was performed in animals randomized to AF groups to prevent tachycardiomyopathy as a consequence of AF induction\textsuperscript{19, 29}. Ventricular backup pacing was provided via the implanted pacemakers. Subsequently, 24 pigs were randomized to an AF group \( (n = 12) \) or a SR control group \( (n = 12) \) with deactivated pacemakers (Fig. 3 A). In the AF group, AF was induced by right atrial burst stimulation. Both study cohorts were divided into a treatment arm \( (n = 5–6) \) with daily doxapram administration \( (2 \text{ mg/kg body weight } \textit{i.v.}) \) and a control arm with sham treatment \( (0.9 \% \text{ NaCl solution}; \ n = 5–6) \). During the 14 d follow-up period, atrial burst stimulation was alternated with pacing-free intervals for automatic rhythm assessment by the pacemakers. A programmable biofeedback algorithm was implemented in the pacing devices that interrupted burst pacing when endogenous AF was detected. At the end of the observation period AF pigs underwent electrical cardioversion. Echocardiography and electrophysiological studies were repeated, and isolated right-atrial cardiomyocytes of all study pigs were subjected to patch-clamp measurements (Fig. 3 A). Additional hemodynamic and echocardiographic characteristics of the study pigs, obtained on day 0 and day 14 are given in Online Table I. Representative surface ECGs, recorded on day 14 are depicted in Fig. 3 B and P waves are highlighted with black dots. Again, doxapram treatment did not result in significant surface ECG changes. TASK-1 inhibition by chronic doxapram treatment did not significantly affect the function of the sinus node (Fig. 3 C). Further analyses of ECG parameters and representative intracardiac recordings of the rhythm state by the implanted devices are shown in Online Figure IV - V. No significant changes of the sinus node recovery times (SNRTs)
measured after 30 s of overdrive suppression and the corrected sinus node recovery times (cSNRT) were observed. Furthermore, sinoatrial conduction times (SACT), measured by applying the Narula\textsuperscript{30} or Strauss\textsuperscript{31} method, remained unchanged (Fig. 3 C).

A mild trend towards an increased AV node conduction was observed in case of continuous treatment with doxapram (Fig. 3 D). The Wenckebach point, the time of AV nodal 2:1 conduction, and AV nodal effective refractory periods (AVNRP), measured at BCLs of 500 ms and 400 ms, were slightly shortened under doxapram treatment (Fig. 3 D). Notably, animals in the AF group received AV node ablations. Therefore, no measurements of AV nodal conduction could be performed in this group.

As expected, AERPs were significantly shortened in untreated AF pigs compared to SR pigs (Fig. 3 E). This observation is consistent with the atrial electrical remodelling, that is also observed in AF patients. Daily doxapram administration increased AERPs to levels observed in the SR control group, reflecting class III antiarrhythmic effects (Fig. 3 E). No significant changes in VERPs were observed under continuous doxapram treatment again indicating absence of off-target effects at the level of ventricular electrophysiology (Fig. 3 F). After 14 d, animals in the AF group without treatment presented AF in 95 % of the analysed surface ECG recordings (Fig. 3 G). In contrast, AF animals receiving daily doxapram \textit{i.v.} treatment displayed AF in only 1 % of the recorded ECGs ($N = 5$ animals; $P < 0.0001$). Thus, long-term doxapram treatment successfully prevented tachypacing induced atrial remodelling.

Measurements of doxapram plasma levels in blood samples of AF and SR animals taken before daily doxapram treatments indicate an initial saturation phase followed by a plateau phase that is reached 5 to 7 d after start of the continuous treatment (Fig. 3 H).
7.5 Effects of chronic doxapram treatment on cellular electrophysiology of atrial cardiomyocytes

Consecutively, effects of pharmacological TASK-1 inhibition by doxapram on atrial cellular electrophysiology were studied in isolated atrial cardiomyocytes from AF and SR pigs. Patch-clamp experiments were performed in the whole-cell configuration. A microscope image of an isolated porcine atrial cardiomyocyte is presented in Fig. 4 A. Representative isolated TASK-1 currents (i.e. differences in currents, recorded under control conditions and after application of 200 nM A293) of the different experimental groups are shown in Fig. 4 B and C. A293-sensitive K\(^+\) current densities were activated at potentials >-20 mV and showed Goldman-Hodgkin-Katz rectification, which is typical for K\(_{2P}\) channels (Fig. 4 D to F). AF-associated upregulation of TASK-1 resulted in a 8.6-fold upregulation of TASK-1 current densities in comparison to SR controls (\(P = 0.024\); \(n / N = 18–24 / 4–6\); \(n\), number of cells; \(N\), number of pigs; Fig. 4 G). Daily i.v. doxapram treatment of AF pigs attenuated TASK-1 current upregulation by 85.2 \% (\(P = 0.15\); \(n / N = 24–26 / 4–6\)) whereas no relevant changes in TASK-1 currents were observed between the SR control animals and SR animals under doxapram treatment (\(P = 0.9\); \(n / N = 14–18 / 4–6\) animals). Resting membrane potentials did not differ significantly among the 4 groups (Online Figure VI).

Atrial cardiomyocyte action potentials (APs) were studied under current-clamp conditions in isolated cells from all four study cohorts. Representative recordings are shown in Fig. 4 H–I. In untreated AF animals APDs at 50 % repolarization (APD\(_{50}\)) were 97.2 ± 10.9 ms (\(n / N = 6 / 4\)) compared to 158.3 ± 14.3 ms in the SR control group (\(n / N = 6 / 4\); \(P = 0.0075\); Fig. 4 J). Under daily doxapram treatment, AF animals displayed APD\(_{50}\) values comparable to SR controls (157.2 ± 4.7 ms; \(n / N = 17 / 4\); Fig. 4 J). Finally, TASK-1 inhibition in SR animals did not cause significant modulation of APD\(_{50}\) values (171.4 ± 10.4 ms; \(P = 0.38\) vs SR control; \(n / N = 11 / 3\); Fig. 4 J). Similar results were obtained for APD\(_{90}\) measurements (Fig. 4 K).
KCNK3 qPCR expression analysis among atrial cardiomyocytes, non-myocytes and cultured atrial fibroblasts points towards cardiomyocyte specific expression of TASK-1 (Online Figure VII).

7.6 Computational modelling of the doxapram effects on atrial electrophysiology in a single-cell and a tissue model

Computational modelling at single-cell and 2-dimensional tissue levels was employed to directly probe the electrophysiological and antiarrhythmic effects of doxapram-mediated TASK-1 inhibition. Our recent human atrial cardiomyocyte model with TASK-1 formulation was fitted to the experimentally obtained current-voltage relationship of human and porcine TASK-1 (Fig. 5 A, left and middle panels), and the concentration-dependent effect of doxapram on TASK-1 was incorporated (Fig. 5 A, right panels). The appropriate concentration of doxapram to simulate long-term (14 d) treatment was determined based on the TASK-1 measurements from the 4 experimental groups shown in Fig. 5. A simulated concentration of 1 µmol/l doxapram reproduced the experimentally observed reduction in TASK-1 (Fig. 5 B).

APD prolongation in the human atrial cardiomyocyte model with AF-related remodelling in response to acute application of 1 µmol/l doxapram was smaller than observed with long-term treatment in pigs. However, when combining the acute inhibition of TASK-1 with a reduction in AF-related remodelling, consistent with the experimentally observed antiarrhythmic effects of doxapram, the electrophysiological phenotype of long-term doxapram treatment could be closely reproduced (Fig. 5 C). Finally, the antiarrhythmic effect of such long-term doxapram treatment was investigated in homogeneous 2-dimensional tissue simulations. Reentry was induced using an S1S2 pacing protocol with different coupling intervals in virtual tissue with and without AF-related remodelling in the absence or presence of the electrophysiological effects of long-term doxapram treatment (Fig. 5 D, left panels). The resulting vulnerable windows with the duration of reentry induced for each S1S2 interval summarize the inducibility
and stability of reentry and the sum of reentry durations over all \( S_1 S_2 \) intervals provides an indication of total arrhythmogenic vulnerability. As expected, the arrhythmogenic vulnerability was considerably larger in the presence of AF-related remodelling (Fig. 5 D, right). Importantly, simulated long-term doxapram treatment reduced reentry duration to values, observe in control animals. Similar results were obtained in the presence of 100 \( \mu \)M doxapram, assuming selective TASK-1 inhibition (Online Figure VIII A-B). When simulating potential off-target effects at this high concentration (36.7 % inhibition of rapid delayed-rectifier K\(^+\) current; 28.0 % inhibition of transient-outward K\(^+\) current and 17.3 % inhibition of ultra-rapid delayed-rectifier K\(^+\) current; based on Online Figure II), doxapram-induced repolarization prolongation was increased, resulting in a minor augmentation of antiarrhythmic effects in tissue simulations (Online Figure VIII C-D). Together, these data support the pronounced antiarrhythmic effect of long-term doxapram treatment.

**7.7 Effects of doxapram on human atrial cardiomyocytes isolated from SR and cAF patients**

Finally, our findings regarding rhythm control in pigs with induced AF were evaluated in tissue samples from AF patients. To this end, atrial cardiomyocytes isolated from patients with cAF or SR were exposed to doxapram. Tissue samples were taken from patients undergoing open heart surgery for valve repair, replacement or coronary artery bypass grafting (Online Table II). A microscope image of an isolated human atrial cardiomyocyte is shown in Fig. 6 A. Representative doxapram-sensitive background potassium currents from voltage-clamp recordings using the patch-clamp technique in whole-cell configuration are shown in Fig. 6 B. Similar to porcine atrial cardiomyocytes, Goldman-Hodgkin-Katz rectification was observed (Fig. 6 C). Doxapram-sensitive potassium background currents recorded from cAF cells were 2.9-fold higher than in SR controls (\( P = 0.02; n / N = 6–15 / 4–8; \) Fig. 6 D).
Collectively, these results point towards class III antiarrhythmic effects of doxapram in native human atrial cardiomyocytes which were even enhanced in cells isolated from AF patients.
8. Discussion

8.1 Antiarrhythmic potential of doxapram

In the development of novel antiarrhythmic drugs, atrial selectivity is essential to prevent potential life-threatening side effects on ventricular electrophysiology\(^4\). In the human heart, TASK-1 channels are predominantly expressed in the atria\(^7\). Such atrial specificity was also observed in domestic pigs\(^{19, 29}\), but not in small animals (e.g. mice)\(^{28}\). Upon heterologous expression of TASK-1 in *Xenopus laevis* oocytes, doxapram has a similar affinity for human and porcine orthologs with IC\(_{50}\) values of 0.88 \(\mu\)M and 0.93 \(\mu\)M, respectively. Similar results were obtained in patch-clamp experiments performed on mammalian cells, with IC\(_{50}\) values of TASK-1 for doxapram inhibition in the single-digit micromolar range\(^{23}\).

Taking the substantial costs and the long timespan associated with drug development into account, repurposing of clinically established drugs with known tolerability becomes an attractive option\(^{33}\). Utilization of the FDA- and EMA-approved drug doxapram may therefore accelerate the evaluation of TASK-1 as a promising target for AF therapy in first clinical trials.

Our present data provides a proof-of-concept that blockade of atrial TASK-1 currents by doxapram exerts class III antiarrhythmic effects *in vivo* sufficient for cardioversion as well as rhythm control of AF induced in pigs. Pharmacological inhibition of atrial TASK-1 currents using the respiratory stimulant drug doxapram facilitated pharmacological cardioversion of induced AF episodes through prolongation of atrial refractoriness. Furthermore, long-term doxapram treatment resulted in rhythm control in a clinically relevant large animal model of persistent AF. No relevant side effects were observed on the basis of ventricular electrophysiological parameters. At a cellular level, doxapram treatment prevented upregulation of TASK-1 currents and APD shortening in isolated porcine atrial cardiomyocytes. The effects of TASK-1 current inhibition and APD prolongation could be
reproduced and mechanistically explained using a computational model of an atrial cardiomyocyte. Multicellular model simulations further replicated the effects on AERPs, confirmed the resulting antiarrhythmic efficacy of doxapram and extrapolated our findings to a broad range of experimental conditions.

Additionally, our study confirms the role of TASK-1 current upregulation in AF-related electrical remodelling, and its implication in shortening of the atrial APD and AERP. Our observations are in line with previous findings in human atrial cardiomyocytes, isolated from AF patients. Enhanced atrial repolarizing K⁺ currents and shortening of refractoriness represent classical characteristics of AF-associated electrical remodelling, promoting reentry and perpetuation of the arrhythmia. In this context, TASK-1 inhibition might serve as a mechanism-based antiarrhythmic concept.

While TASK-1 channels display atrial-specific expression patterns within the human heart, TASK-1 and closely related TASK-3 channels are expressed in several other organ systems where they control numerous important physiological processes. TASK-1 expression was reported in the brain, lung, liver, kidney, adrenal gland, pancreas, placenta, ovary, prostate, small intestine and chondrocytes. Animal models pointed towards a role of TASK-1 currents in mediating anaesthetic regulation of neuronal activity, breathing stimulation, regulation of thermogenesis, pulmonary vascular tone, immunoresponse, aldosterone secretion and tumorigenicity. Thus, when employing inhibitors of TASK-1 channels for treatment of AF, care has to be taken not to interfere with other organ functions. Following an initial saturation phase, doxapram plasma levels of our study pigs reached a plateau phase in the range of 0.1-1 µg/ml. When applied as a respiratory stimulant, therapeutic plasma levels of doxapram were reported to reach 2 µg/ml.
In dogs, an acute increase in systemic blood pressure (10-20 mmHg) was reported after doxapram treatment, accompanied by an increase (~25%) in cardiac output. A clinical study in patients, receiving doxapram following thoracic surgery found no change in blood pressure or hemodynamics. The observation that doxapram, applied during right heart catheterization of patients suffering from chronic bronchitis, results in a ~10 mmHg increase in pulmonary artery pressure could be attributed to TASK-1 channel expression in pulmonary artery smooth muscle cells. Another potential side effect described in the use of doxapram in neonatology is an increased incidence of hypokalemia, which is associated with increased aldosterone levels and is reversible after discontinuation. This side effect could also be explained by TASK channel inhibition, as the knockout of TASK channel genes in mice was shown to result in a phenotype of primary hyperaldosteronism.

Doxapram has been routinely employed as a respiratory stimulant in human and veterinary medicine for over half a century. However, to our best knowledge, the present study is the first systematic characterization of its antiarrhythmic properties, because previous studies only reported effects of doxapram on the QT interval or on surface ECG parameters. Early studies in anaesthetized dogs and patients with structural heart diseases showed an increase of ventricular ectopy after doxapram treatment. It remains, however, uncertain whether this ectopy was primarily caused by electrophysiological effects of doxapram or by an increased sympathoadrenergic drive, increased cardiac output or an increased respiratory drive. Interestingly, De Villies et al. reported the development of a second-degree atrioventricular block in three cases of premature infants treated with high doses of doxapram (1.47 mg/kg/h or 15–18.5 mg/kg four times per day) that could be related to adverse effects on cardiac conduction. Furthermore, occurrence of junctional rhythms was described in dogs treated with high doxapram doses (5 mg/kg). In comparison to the studies by De Villies et al. and...
Maillard et al., however, our experiments showed no significant effects of doxapram on ventricular repolarization\textsuperscript{47, 49}.

Current pharmacological therapies for rhythm control in AF patients as well as cardioversion are still limited. Due to its atrial-specific expression, upregulation in AF, and its functional connection to the APD length, TASK-1 might be a promising target for AF suppression. In this study, we observed that doxapram, a specific TASK-1 inhibitor, could be successfully administered for cardioversion and rhythm control of AF in pigs. Our experiments in porcine atrial cardiomyocytes showed an upregulation of TASK-1 in AF, resulting in elevated TASK-1 currents that could be inhibited by doxapram treatment, resulting in values, observed among SR cardiomyocytes. Due to the indication of doxapram as a breathing stimulant, its antiarrhythmic effect might be studied in sleep apnea patients that frequently suffer from AF. Based on the preclinical results in this animal study, we started the DOCTOS trial (doxapram conversion to sinus rhythm; EudraCT No: 2018-002979-17) in 2019 as the first clinical trial evaluating the efficacy of doxapram for AF cardioversion.

\textbf{8.2 Potential limitations}

The porcine animal model of right atrial tachypacing-induced AF might differ from clinically observed spontaneous AF, regarding the pathophysiological mechanism of arrhythmogenesis. Previous electrophysiological studies, however, showed similar functional and molecular characteristics of the porcine AF model to AF in human\textsuperscript{18, 25}. Further, AF induction and antiarrhythmic drug therapy were started in parallel therefore, the model may reflect the clinical situation of a patient with a mild atrial substrate at the threshold of paroxysmal to persistent AF, rather than patients with already persistent AF.
Another potential limitation could result from interactions between atrial electrophysiology, doxapram treatment, respiratory rate, blood pressure, or sympathoadrenergic drive. To comprehensively quantify the effects of doxapram on cardiac electrophysiology and to appreciate the role of the autonomic nervous system in the pathophysiology of AF, electrophysiological and hemodynamic measurements were performed without complete autonomic blockade. Even though contribution of TASK-1 to fibroblasts appeared limited (Online Figure VII), it remains uncertain whether doxapram has an additional beneficial effect on atrial remodelling beyond its primary electrical effects through modulation of atrial inflammation via TASK-1 channel inhibition in immunocytes or through interaction of central nervous TASK-1 channels. Due to regulations on animal protection, the treatment duration of 14 d was relatively short and sample sizes used in this study were relatively small.

AV node ablation was performed in the AF group to prevent tachycardiomyopathy because left ventricular ejection fraction was identified as an important remote regulator of atrial TASK-1 currents. Rapid conduction through the AV node is, however, part and parcel of atrial arrhythmogenesis in patients with heart failure. It has to be taken into account that this element of the clinical picture of AF is not taken into consideration in the animal model that was used in this study. It further has to be acknowledged that not all study groups underwent AV node ablation, as we aimed to assess the effects of doxapram on AV nodal conduction.

8.3 Conclusion
Pharmacological suppression of atrial TASK-1 potassium channels prolonged atrial refractoriness with no effects on ventricular repolarization, resulting in atrial-specific class III antiarrhythmic effects. In our preclinical pilot study using a clinically relevant large animal model, the respiratory stimulant doxapram was successfully administered for cardioversion of acute episodes of paroxysmal AF as well as rhythm control of persistent AF. Further clinical
trials are needed to evaluate the effects of doxapram in AF patients. Finally, experiments of
this study served as the basis for preparing the first clinical trial of a TASK-1 inhibitor in AF
patients, the DOCTOS trial, to convert AF.
9. Acknowledgments

We thank Sabine Höllriegel, Patricia Kraft, Katrin Kupser and Lisa Künstler for excellent technical support.

10. Sources of Funding

This work was supported by research grants from the University of Heidelberg, Faculty of Medicine, Heidelberg, Germany [Rahel Goitein-Straus Scholarship and Olympia-Morata Scholarship to C.S.]; the German Center for Cardiovascular Research, Berlin, Germany (DZHK) [Excellence Grant to C.S.; Excellence Program: Postdoc Start-up Grant to F.W.]; the German Cardiac Society (DGK), Dusseldorf, Germany [Research Scholarship DGK082018 to F.W.; Otto-Hess Fellowship to F.W. and C.B.]; the German Heart Foundation /German Foundation of Heart Research, Frankfurt [F/15/18 to F.W., F/41/15 to C.S., Kaltenbach Scholarship to A.B. and F.W.]; the Joachim-Herz Foundation, Hamburg, Germany [PostDoc Addon-Fellowship to F.W.] and from the German Research Foundation (DFG), Bonn, Germany [SCHM 3358/1-1 to C.S., SFB 1425 to C.S.].

11. Data availability statement

The data underlying this article are available in the article and in its online supplementary material.

12. Author Contributions

F.W. and C.S. designed, performed and analysed the majority of experiments in this study and co-wrote the manuscript. N.D., G.v.L., and S.K. provided valuable support on experimental design, data analysis and interpretation. C.B., F.W., M.Kr., and C.S. performed the large animal experiments. F.W., M.Kr., N.J., A.P., S.R. and A.Bü. performed and analysed the two electrode
voltage-clamp experiments in *Xenopus laevis* oocytes and patch-clamp experiments in human atrial cardiomyocytes. A.Bü. performed and visualized the molecular docking simulations. X.-B.Z., I.E.-B., X.L., S.L., I.A., and M.B. performed patch-clamp experiments in isolated porcine cardiomyocytes. M.Kr., K.I.F., A.Bl. and W.E.H. performed the MS doxapram plasma level analysis. H.S. and J.H. performed the computational simulations. U.T., J.K., R.A. and M.Ka. contributed to human tissue sample acquisition. M.Ka., N.D., G.v.L., M.B., S.K., J.H., W.E.H., and H.A.K. supported manuscript and figure preparation. C.S. supervised the project. All authors have approved the submitted version of the manuscript and have agreed to be personally accountable for their contributions.

13. Disclosures

F.W., H.A.K., and C.S. have filed a patent application for *KCNK3*-based gene therapy for cardiac arrhythmia. F.W., N.D., W.E.H, H.A.K., and C.S. have filed a patent application for pharmacological TASK-1 inhibition in treatment of atrial arrhythmia. The remaining authors have reported that they have no relationships relevant to the content of this paper to disclose.
14. Supplemental Materials

2 Expanded Materials & Methods
3 Online Figures I-VIII
4 Online Table I-II
15. References


23. Cunningham KP, MacIntyre DE, Mathie A, Veale EL. Effects of the ventilatory stimulant, doxapram on human TASK-3 (KCNK9, K_{2P}9.1) channels and TASK-1 (KCNK3, K_{2P}3.1) channels. *Acta Physiol (Oxf)* 2020;228:e13361.


16. Figures

Graphical abstract
Figure 1: Pharmacological effects of doxapram on TASK-1 potassium channels
(A) Left: hTASK-1 crystal structure\textsuperscript{26}. Right: homology model of pTASK-1 based on the respective crystal structure. Differences in hTASK-1 and pTASK-1 amino acid sequence are indicated in red. (B) Chemical structure of monohydrated pyrrolidinone derivative doxapram. (C) Simulation of doxapram docking to the inner channel pore of pTASK-1. Excerpts illustrate the interactions of doxapram with pore-associated amino acids. (D to I) Human and porcine TASK-1 ion channel subunits, heterologously expressed in \textit{Xenopus laevis} oocytes, are inhibited by doxapram (10 µmol/l) to a similar extent. (D and G) Representative current traces of hTASK-1 (D) and pTASK-1 (G) before (CTRL) and after 10 µmol/l doxapram. (E and H) Current-voltage relationships of human (E) and porcine (H) TASK-1 before (CTRL) and after doxapram \((n = 3–4)\). (F and I) Concentration–response relationships for the effect of doxapram, on human (F) and porcine (I) TASK-1 \((n = 3–24)\). (J) TASK-specificity of doxapram among atrial potassium channels assessed in oocytes \((n = 4–8)\). Significant current reduction was observed in TASK-1 and noncardiac TASK-3 subunits. (J) Representative current traces recorded under control conditions (grey) and after doxapram (5 µM, 30 min) by application of voltage pulses from -80 mV to +40 mV for TASK-1, TASK-3, TASK-4, TREK-1, MaxiK, Kv1.4, Kv1.5, Kv2.1, and Kv4.3, pulses from -160 mV to 0 mV for Kir2.1 and Kir3.1/Kir3.4 or a double step from -80 mV to +20 mV followed by a second step to -40 mV for hERG. For Na\textsubscript{V}1.5 currents, the voltage was stepped from a holding potential of -80 mV to -30 mV, utilizing a digital leak subtraction with a p over n protocol of n = 4. For Cav1.2 voltage was stepped from -80 mV to +10 mV for 1 s, sweep time interval was 30 s. (K) Relative current inhibition levels are compared. Data for Cav1.2 recordings were corrected for rundown. Dashed lines: zero current level. Data are presented as mean ± SEM. *, \(P < 0.05\) vs respective CTRL for Student’s t-tests.
Figure 2: Acute effects of doxapram on atrial electrophysiology, insights from a porcine large animal model
Doxapram plasma concentrations measured in 60 min intervals after intravenous administration of doxapram, 2 mg/kg body weight (BW; n = 3). (B) Representative surface electrocardiograms (ECGs) recorded in pigs before and 10 min after doxapram administration, 2 mg/kg BW (n = 7 experiments performed on N = 4 pigs). (C) Upon administration of doxapram a non-significant trend towards faster heart rates could be observed (n / N = 7 / 4). (D) Systolic (RR$_{sys}$), diastolic (RR$_{diast}$), and mean (RR$_{mean}$) arterial blood pressure levels were not significantly changed by doxapram treatment (n / N = 7 / 4). (E) Administration of doxapram significantly prolonged the atrial effective refractory periods (n / N = 7 / 4). (F–H) After induction of atrial fibrillation (AF) atrial rhythm was monitored for 5 min. When AF persisted different doses of doxapram were intravenously administered and time to cardioversion was measured. (F) Surface and intracardiac ECG recordings. The magnification visualizes time of cardioversion to sinus rhythm marked with an asterisk. (G) Time to cardioversion of AF after infusion of doxapram (n / N = 17 / 17). (H) Doxapram dosages that were not effective (white circles) or effective for cardioversion (blue dots). Data are shown as mean ± SEM. *, P < 0.05; **, P < 0.01; ***, P < 0.001 vs baseline for Student’s t-tests. Where no asterisks are presented, no statistically significant difference was observed in Student’s t-tests.
Figure 3: Safe and effective treatment of persistent atrial fibrillation (AF) using doxapram
**in a porcine disease model**

(A) Experimental protocol: subsequent to an electrophysiological (EP) investigation and pacemaker implantation, 20 pigs were randomized to AF induction or a sinus rhythm (SR) control (CTRL) group. AF and SR groups were divided into subgroups receiving intravenous doxapram or sham treatment. (B) Surface electrocardiograms (ECGs) from all 4 subgroups on day 14 (*left panel*). Black dots indicate P waves. Scale bar (bottom right) denotes 200 ms. **Right:** mean surface ECG parameters. PQ intervals could not be measured after AV node ablation in the AF groups. (C) SNRT, cSNRT and SACT. (D) AV nodal effective refractory periods measured in SR animals. (E) Atrial effective refractory periods (ERPs) were significantly reduced in the AF-induction group and could be restored by doxapram treatment. *, $P < 0.05$; **, $P < 0.01$ vs 14 d SR CTRL in Student’s t-tests. (F) No statistically significant differences of ventricular ERPs were observed. (G) AF burden (i.e. diagnosis of AF in daily surface ECGs in relation to the cumulative number of surface ECGs, documented during the 14 d follow-up period) was significantly reduced by doxapram treatment. ***, $P < 0.001$ vs 14 d AF CTRL in Student’s t-tests. (H) Plasma levels of doxapram in AF and SR animals in daily blood samples. Where no asterisks are presented, no statistically significant difference was observed in Student’s t-tests.
Figure 4: Patch-clamp recordings of atrial TASK-1 currents and action potentials (APs) of sinus rhythm (SR) and atrial fibrillation (AF) pigs during long-term doxapram treatment

(A) Isolated porcine atrial cardiomyocyte attached to a patch-clamp micropipette (scale bar: 25 µm). (B and C) TASK-1 current densities recorded from atrial cardiomyocytes from AF or SR pigs after 14 d. The pulse protocol used in voltage-clamp measurements is depicted in panel (B). (D to F) Dependence of mean step current densities on test potentials for the treatment and control groups (n = 11-26 cells, from N = 4–6 different animals per group). (G) Comparison of average A293-sensitive current densities, quantified at the end of +20 mV pulse. (H to I) Representative AP recordings of atrial cardiomyocytes. (H to I) Comparison of corresponding average AP durations at 50 % repolarization (APD₅₀; J) or 90 % repolarization (APD₉₀; K) of experimental groups (n = 6–17 cells, isolated from N = 3–4 animals per group). Data are shown as mean ± SEM. Dashed lines indicate zero current and potential levels.

*, P < 0.05; **, P < 0.01 from Student’s t-tests.
Figure 5: In silico analysis of the effects of doxapram on human atrial electrophysiology and arrhythmogenesis

(A) Voltage dependence of human and porcine TASK-1 in the absence (dark symbols/lines) or presence (light symbols/lines) of 10 µmol/l doxapram (left and middle), concentration-dependent inhibition of TASK-1 by doxapram (right) in experiments (symbols; data from Fig. 1) and model (lines). (B) Voltage dependence of TASK-1 currents in sinus rhythm (SR) and atrial fibrillation (AF) pigs with sham treatment (CTRL) or treated with doxapram (symbols; data from Fig. 4) and in the human atrial cardiomyocyte model with pTASK-1 (solid lines) or hTASK-1 (dashed lines). (C) Validation of action potential (AP) properties of the computer model with human or porcine TASK-1 without (SR) or with AF-related remodelling in the absence (CTRL) or presence of the electrophysiological effects of long-term (14 d) doxapram treatment (simulated as TASK-1 inhibition by 1 µM doxapram and 75 % reduction in AF-related electrical remodelling). Bar charts show relative changes in AP duration at 50 % and 90 % of repolarization (APD_{50} and APD_{90}) in models compared to experimental data from Fig. 4. (D) Snapshots of simulated reentry initiated by an S_1S_2 interval of 130 ms in 2-dimensional human atrial tissue models with or without AF-related remodelling in the absence (CTRL) or presence of the electrophysiological effects of long-term doxapram treatment (Doxa; left), and the vulnerable windows with reentry duration for different S_1S_2 interval together with the sum of all reentry durations, representing total arrhythmogenic risk.
Figure 6: Effects of doxapram on human atrial cardiomyocytes from SR controls and AF patients

(A) Isolated human atrial cardiomyocyte (scale bar: 25 µm). (B to C) Potassium currents in human atrial cardiomyocytes after 100 µmol/l doxapram. (B) Representative current recordings for cardiomyocytes from SR and chronic AF patients. (C) Dependence of mean step current densities on the respective test pulse potentials (n = 6–15 cells, from 4–8 patients per group; no statistical significance in Bonferroni corrected Mann-Whitney tests).

(D) Comparison of mean doxapram-sensitive current densities at +20 mV. Data are shown as mean ± SEM. Zero current levels are indicated by dashed lines. *, P < 0.05 from Mann-Whitney test.